

Application No.: 10/717,677
Response dated: August 10, 2007
Reply to Office Action dated: May 18, 2007

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (withdrawn-currently amended) A method of culturing human mammalian embryonic stem (ES) cells with reduced differentiation comprising:
 - a) growing the human ES cells in culture on a flexible solid porous matrix without conditioned media and in the absence of fibroblast feeder cells; and
 - b) applying an effective amount of periodic strain on the flexible matrix to stretch the matrix and the human ES cells thereon, such that the human ES cells proliferate and exhibit reduced differentiation relative to human ES cells not subjected to periodic strain.
2. -3. canceled.
4. (withdrawn) The method of Claim 1 wherein the cell differentiation is eliminated.
5. (withdrawn) The method of Claim 1 wherein the cells are grown on Matrigel™ using BioFlex® untreated culture plates.
6. (withdrawn) The method of Claim 1 wherein the cells are grown without the presence of cross-species biological material.
7. (withdrawn) The method of Claim 1 wherein the flexible matrix is Matrigel™.
8. (withdrawn) The method of Claim 1 wherein the strain is mechanically produced.

Application No.: 10/717,677
Response dated: August 10, 2007
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9. (withdrawn) The method of Claim 1 wherein the flexible matrix is stretched using vacuum pressure.

10. (withdrawn) The method of Claim 1 wherein the strain exerted on the flexible matrix is at least about 5%.

11. (withdrawn) The method of Claim 1 wherein the flexible matrix undergoes at least about 6 stretches per minute.

12. (withdrawn) The method of Claim 1 wherein the mechanical strain is from oscillatory stretching of the flexible matrix surface.

13. (currently amended) A cell culture composition comprising:
~~mammalian human embryonic stem (ES) cells~~ in culture without conditioned media or fibroblast feeder cells;
a flexible solid porous matrix, wherein the cells are on the matrix; and
an apparatus for applying an effective amount of periodic strain on the flexible matrix to stretch the matrix and the ~~human ES~~ cells thereon, such that the ~~human ES~~ cells proliferate and exhibit reduced differentiation relative to ~~human ES~~ cells not subjected to periodic strain.

14.-15. canceled.

16. (previously presented) The culture of Claim 13 wherein the cell differentiation is eliminated.

17. (previously presented) The culture of Claim 13 wherein the cells are grown on Matrigel™ using BioFlex® untreated culture plates.

18. (previously presented) The culture of Claim 13 wherein the cells are grown without the presence of cross-species biological material.

Application No.: 10/717,677
Response dated: August 10, 2007
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19. (previously presented) The culture of Claim 13 wherein the flexible solid porous matrix is Matrigel™.

20. (previously presented) The culture of Claim 13 wherein the strain is mechanically produced.

21. (previously presented) The culture of Claim 13 wherein the flexible matrix is stretched using vacuum pressure.

22. (previously presented) The culture of Claim 13 wherein the mechanical strain is from oscillatory stretching of the flexible matrix surface.

23. (previously presented) The culture of Claim 13 wherein the strain exerted on the flexible matrix is at least about 5%.

24. (previously presented) The culture of Claim 13 wherein the flexible matrix undergoes at least about 6 stretches per minute.

25. (withdrawn- currently amended) A method of culturing mammalian human embryonic stem (ES) cells with reduced differentiation comprising:

- a) growing the human ES cells in culture on a flexible solid porous matrix without conditioned media and in the absence of fibroblast feeder cells; and
- b) applying an effective amount of periodic strain on the human ES cells, such that the human ES cells proliferate and exhibit reduced differentiation relative to human ES cells not subjected to periodic strain.

26. (new) A cell culture composition comprising:
undifferentiated human stem cells in culture without conditioned media or fibroblast feeder cells, wherein the stem cells are defined by the positive expression of Oct4 and SSEA-4 cell surface markers;

a flexible solid porous matrix, wherein the cells are on the matrix; and

Application No.: 10/717,677
Response dated: August 10, 2007
Reply to Office Action dated: May 18, 2007

an apparatus for applying an effective amount of periodic strain on the flexible matrix to stretch the matrix and the undifferentiated human stem cells thereon, such that the human stem cells proliferate and exhibit reduced differentiation relative to undifferentiated human stem cells not subjected to periodic strain.

27. (new) The method of Claim 26 wherein the undifferentiated human stem cells are also immuno-positive for presence of alkaline phosphatase.

28. (new) A method of culturing undifferentiated human stem cells with reduced differentiation comprising:

a) growing the undifferentiated human stem cells in culture on a flexible solid porous matrix without conditioned media and in the absence of fibroblast feeder cells, , wherein the stem cells are defined by the positive expression of Oct4 and SSEA-4 cell surface markers; and

b) applying an effective amount of periodic strain on the flexible matrix to stretch the matrix and the undifferentiated stem cells thereon, such that the undifferentiated cells proliferate and exhibit reduced differentiation relative to undifferentiated human stem cells not subjected to periodic strain.